PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 489/04, C07B 61/00, A61K 31/40 — A1

(11) International Publication Number: WO 99/12935

(43) International Publication Date: 18 March 1999 (18.03.99)

(21) International Application Number: PCT/EP98/05744

(22) International Filing Date: 7 September 1998 (07.09.98)

(30) Priority Data:
9719161.3 9 September 1997 (09.09.97) GB

(71) Applicant (*for all designated States except US*): GLAXO GROUP LIMITED [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): COOTE, Steven, John [GB/GB]; (GB). DOWLE, Michael, Dennis [GB/GB]; (GB). FINCH, Harry [GB/GB]; (GB). HANN, Michael, Menteith [GB/GB]; (GB). KELLY, Henry, Anderson [GB/GB]; (GB). MACDONALD, Simon, John, Fawcett [GB/GB]; (GB). PEGG, Neil, Anthony [GB/GB]; (GB). RAMSDEN, Nigel, Grahame [GB/GB]; (GB). WATSON, Nigel, Stephen [GB/GB]; Glaxo Wellcome plc, Gunnels Wood Road, Stevenage, Hertfordshire SG1 2NY (GB).

(74) Agent: TEUTEN, Andrew, J.; Glaxo Wellcome plc, Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PYRROLOPYRROLIDINE DERIVATIVES AND THEIR USE AS SERINE PROTEASE INHIBITORS

(57) Abstract

There is provided according to the invention an inhibitor of a serine protease enzyme which is a substituted derivative of trans–hexahydropyrrolo[3,4–b]pyrrol–2–one.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

PYRROLOPYRROLIDINE DERIVATIVES AND THEIR USE AS SERINE PROTEASE INHIBITORS

This invention relates to a new class of chemical compounds and their use as inhibitors of serine protease enzymes. These compounds are useful as pharmaceuticals and we provide processes for preparing them and formulations containing them.

Serine proteases are a class of proteolytic enzymes characterised by having at the active site a serine residue which interacts with the carbonyl carbon of a peptide bond to cleave the peptide bond via an acyl enzyme intermediate. Under the conventional residue numbering based on homology with the serine protease enzyme chymotrypsin, the active site serine is generally numbered Ser-195. Most members of the family of serine proteases have a histidine and an aspartic acid residue in the active site (numbered His-57 and Asp-102 based on chymotrypsin) which activate the serine hydroxyl group to attack the scissile peptide carbonyl. In a small number of enzymes (notably Herpes virus proteases) the role of Asp-102 is taken by a further histidine residue (which is His-157 in cytomegalovirus protease). Residue mutation studies have shown these three residues to be essential for activity and they are conventionally referred to as the "catalytic triad".

Although the mechanism of hydrolysis of peptide bonds by serine proteases is believed to be similar for all enzymes in the family, it is well known that their substrate specificities differ dramatically. In general, specificity is shown for peptide bonds which have a particular moiety α to the scissile peptide carbonyl which in conventional nomenclature is said to be in the P_1 position and to occupy the S_1 specificity subsite (see Schlecter and Berger (1967) Biochem

Biophys Res Common 27 157). For example, the preferred substrate for thrombin is a peptide containing a basic residue (e.g. arginine i.e. the moiety $(CH_2)_3NHC(=NH)NH_2$) is in the P_1 position) whereas the preferred substrate for elastase is a peptide containing a valine residue (i.e. the moiety $CH(CH_3)_2$ is in the P_1 position).

The X-ray crystal structures of a substantial number of serine protease enzymes have become available in recent years. It can be concluded in explanation of the above observations, that the "catalytic triad" is generally highly conserved in terms of its spacial orientation at the active site and that a major factor in the difference in substrate specificity comes from the shape and character of the S_1 specificity subsite.

Serine proteases are widespread in the human body and abnormal or excessive activity of serine proteases is implicated in a diverse range of diseases and conditions (see "Proteinase Inhibitors", Barrett and Salveson (1986), Elsevier, p56; Drugs Future (1996), 21(8), 811-816; Exp. Opin. Ther. Patents (1997) 7(1) 17-28).

The following enzymes and associated conditions are exemplary:

Neutrophil elastase is found in neutrophil azurophilic granules associated with tissue inflammation and is associated with a number of inflammatory diseases including emphysema, chronic bronchitis and adult respiratory distress syndrome (ARDS).

Members of the blood coagulation cascade (e.g. thrombin, Factor VIIa, Factor Xa, Factor XIa, Factor XIIa) and members of the fibrinolytic cascade (e.g. tissue plasminogen activator and plasmin) are potential targets for treatment of

diseases of the vascular system. For example, thrombin is a potential target for the treatment of thrombosis. Tissue plasminogen activator and plasmin may also be implicated in tumour metastasis.

Tryptase is present in mast cells and inhibitors of tryptase have shown efficacy in models of asthma.

Pancreatic elastase, trypsin and chymotrypsin are associated with digestive disorders such as pancreatitis.

Cathepsin G is associated with emphysema.

Serine proteases are also widespread in human pathogens especially viruses and these provide an attractive target for the treatment of pathogenic diseases and conditions.

For example Herpes viruses encode a serine protease which is crucial for viral replication and is therefore a target for the treatment of conditions caused by these viruses.

The Herpes family of viruses is responsible for a wide range of human infectious diseases including chicken pox and shingles (varicella and Herpes zoster viruses, respectively), cold sores and genital herpes (herpes simplex virus), retinitis, pneumonitis and keratitis (human cytomegalovirus, hCMV), as well as diseases caused by Epstein Barr Virus (EBV), human herpes virus 6 (HHV 6), HHV 7 and HHV 8.

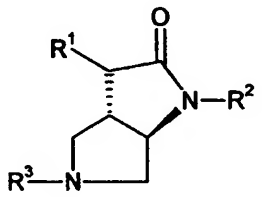
Hepatitis C virus also encodes a serine protease (known as the NS3 serine protease) which is a target for treatment of Hepatitis C virus infection and associated hepatic damage.

It will be appreciated that aside from the enzymes and conditions mentioned above many other serine protease enzymes are known to be suitable targets for pharmaceutical therapy and indeed it can be expected that many more will be identified in the future.

We have now invented a novel chemical class of molecules which are capable of inhibiting a wide range of serine protease enzymes. As such they are of potential value in the treatment of diseases as discussed above.

More particularly, according to the invention, we provide inhibitors of serine protease enzymes which are substituted derivatives of trans-hexahydropyrrolo[3,4-b]pyrrol-2-one.

Most particularly, this invention relates to inhibitors of serine protease enzymes which are compounds of formula I:



(I)

(relative stereochemistry indicated)

wherein R^1 is a moiety adapted to fit in the S_1 specificity subsite of the enzyme;

R^2 is a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and enzyme kinetic properties of the inhibitor;

R^3 is a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and physicochemical properties of the inhibitor; and physiologically acceptable salts and solvates thereof.

Without being limited by theory, we believe that the translactam template of formula I is highly complementary to the active site of serine proteases and the lactam carbonyl mimics the peptide carbonyl of the enzyme's natural substrate. Inhibition of serine proteases by compounds of the invention has been found to be either competitive (reversible) or time-dependent (acylating) depending on the precise enzyme and particular substitution pattern on the translactam template. Time-dependent (acylating) inhibition is believed to occur when attack of the enzyme active site serine on the translactam carbonyl causes opening of the strained lactam ring generating an enzyme acylated at the serine sidechain.

The advantages of our invention reside *inter alia* in that (a) the trans-hexahydropyrrolo[3,4-b]pyrrol-2-one template is completely new and therefore highly desirable in a medicament especially for the treatment of pathogenic conditions which are prone to drug resistance, (b) the trans-hexahydropyrrolo[3,4-b]pyrrol-2-one template may be highly functionalised and is therefore ideal for the specific and selective inhibition of a wide range of different enzymes, (c) the trans-hexahydropyrrolo[3,4-b]pyrrol-2-one template may potentially be functionalised to give (i) high or low metabolic stability and (ii) competitive or time-dependent inhibition as desired.

The determination of the optimum substitution of the derivatives of trans-hexahydropyrrolo[3,4-b]pyrrol-2-one, especially regarding selection of groups R^1, R^2 and R^3 for a particular serine protease enzyme can be made in a conventional manner, namely: (a) by preparation of a number of compounds having sufficient diversity especially in groups R^1, R^2 and R^3, (b) treatment of a

sample of the enzyme in question with a sample of each of the compounds so prepared and (c) determining the extent to which inhibition of the enzyme has occurred.

Assays for enzyme inhibition will generally be well known and in any event will be capable of being performed by a person skilled in the art.

More particularly:

Suitable R^1 groups will fit appropriately in the S_1 specificity subsite of the target enzyme. Choice of group R^1 may be made having regard to the known substrate specificity preferences of the target enzyme, crystallographic information concerning the geometry of the S_1 specificity subsite of the target enzyme and/or empirical determination based on screening data (see for example "Proteinase Inhibitors" Barrett and Salveson (1986), Elsevier, p9 and p59).

When classified by their primary substrate specificity, there are three major types of serine proteinases: elastase-like, trypsin-like and chymotrypsin-like. The differences between these types can be understood in structural terms - see Kraut J (1977) Am. Rev.Biochem. 46 331-358).

For inhibition of neutrophil elastase and elastase-like enzymes, the group R^1 is preferably small and hydrophobic, e.g. C_{2-4}alkyl or C_{2-4}alkenyl, especially propyl or isopropyl, particularly isopropyl.

For inhibition of chymotrypsin-like enzymes (including chymotrypsin and cathepsin G) the group R^1 is preferably large and hydrophobic, e.g. $(CH_2)_{1-2}Ph$, $(CH_2)_{0-2}$cyclohexyl, t-butyl.

Ph represents phenyl or substituted phenyl (e.g. phenyl substituted by C_{1-6}alkyl, halogen). Planar aromatic sidechains (e.g. benzyl) are especially preferred.

For inhibition of trypsin-like enzymes (including trypsin, thrombin, tryptase, Factor VIIa, Factor Xa, Factor XIa, Factor XIIa) the group R^1 is preferably basic e.g.$(CH_2)_{2-4}NHC(=NH)NH_2$, $(CH_2)_{1-2}PhC(=NH)NH_2$, $(CH_2)_{3-5}C(=NH)NH_2$, CH_2(cyclohexyl)NH_2, $(CH_2)_{1-3}(NH)_{0-1}$Het (wherein Het represents a 5 or 6 membered aromatic ring containing 1 or more nitrogen atoms and optionally substituted by amine) or $(CH_2)_{3-5}NH_2$ especially $(CH_2)_4C(=NH)NH_2$ or $(CH_2)_3NHC(=NH)NH_2$, particularly $(CH_2)_4C(=NH)NH_2$.

R^2 will be a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and enzyme kinetic properties of the serine protease inhibitor. Preferably, R^2 will be lactam activating moiety. Suitable activating groups include electron withdrawing groups which may typically (but not exclusively) comprise a SO_2 or CO moiety attached to the lactam nitrogen.

For inhibition of neutrophil elastase, R^2 may represent CHO or SO_2C_{1-6}alkyl and is preferably a group -SO_2Me. For inhibition of thrombin R^2 is preferably a group $CONH(CH_2)_{1-4}Ph$, $SO_2(CH_2)_{0-1}Ph$, -$COOC_{1-4}$alkyl (e.g. -COOMe) or -$CONHC_{1-4}$alkyl (e.g. -CONHMe). These may also be the preferred R^2 for other trypsin-like enzymes.

When R^2 is highly activating we find that the inhibitors act through a time-dependent (acylating) mechanism whereas when R^2 is less activating, the inhibitors may act through a reversible (competitive) mechanism.

When R^2 comprises an SO_2 moiety attached to the lactam nitrogen, the inhibitor is generally time-dependent (acylating). When R^2 comprises a CO moiety attached to the lactam nitrogen, the inhibitor may be time-dependent (acylating) or not depending on the exact nature of R^2. When R^2 represents $COOC_{1-4}$alkyl it is more likely to be time-dependent (acylating) than when R^2 represents $CONHC_{1-4}$alkyl.

R^3 will be a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and physicochemical properties of the serine protease inhibitor. It may also be adapted to optimise other pharmacological properties such as water solubility and oral activity (if desired).

In general, R^3 can vary quite widely and a person skilled in the art would be able to determine from suitable testing if a given R^3 is suitable for the aforementioned purposes or not.

Frequently, an increase in potency is achieved when the R^3 moiety binds at a remote specificity subsite such as S_3, S_4, or S_5 (see "Proteinase Inhibitors", Barrett and Salveson (1986) Elsevier, p6, 69).

We have found that it is often preferred that R^3 comprises a CO, SO_2 or CO.O (especially a CO or SO_2) moiety attached directly to the pyrrolidine nitrogen and is, for example, a group of formula $R^{30}CO$, $R^{30}SO_2$ or $R^{30}OCO$ (especially $R^{30}CO$ or $R^{30}SO_2$).

R^{30} will also be a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and physicochemical properties of the serine protease inhibitor and may represent, for example, alkyl (e.g. C_{1-8}alkyl), alkenyl

(e.g. C_{1-8}alkenyl), aryl, alkylaryl (e.g. C_{1-8}alkylaryl), or alkenylaryl (e.g. C_{1-8}alkenylaryl).

Where used herein, alkyl includes branched and cyclic alkyl. Alkenyl includes branched and cyclic alkenyl.

Aryl includes mono and bicyclic aromatic rings optionally containing heteroatoms, e.g. O, N and S atoms (for example 1 to 4 heteroatoms).

Alkyl, alkenyl, aryl, alkylaryl and alkenylaryl groups may be optionally substituted, e.g. by amine and halogen and optionally interrupted by a heteroatom (e.g. nitrogen or oxygen) or otherwise functionalised.

Amine groups include primary, secondary and tertiary amine groups including cyclic amine.

Thus according to the invention we also provide a method of inhibiting a serine protease enzyme which comprises treating it with a compound of the invention.

We also provide a method of screening for inhibitors of serine proteases which comprises treating a serine protease enzyme with a compound of the invention and determining the extent to which inhibition has occurred.

We also provide a method of identifying an inhibitor of a serine protease enzyme which comprises:

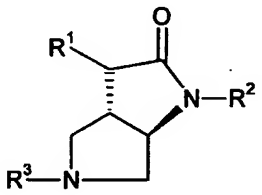
(a) preparation of a number of substituted derivatives of trans-hexahydropyrrolo[3,4-b]pyrrol-2-one;

(b) treatment of a sample of the enzyme in question with a sample of each of the derivatives so prepared; and

(c) determining the extent to which inhibition of the enzyme has occurred.

The extent to which inhibition has occurred may be determined by conventional assay techniques including (but not limited to) chromogenic assays, fluorogenic assays, HPLC and scintillation proximity assays.

In one particularly advantageous method of drug discovery, a library comprising a plurality of substituted derivatives of trans-hexahydropyrrolo[3,4-b]pyrrolo-2-one will be prepared. Preferably the library will comprise a plurality of compounds of formula I



(I)

wherein R^1 is a moiety adapted to fit in the S_1 specificity subsite of the enzyme; R^2 is a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and enzyme kinetic properties of the inhibitor; R^3 is a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and physicochemical properties of the inhibitor; and physiologically acceptable salts and solvates thereof.

The library will, ideally comprise at least 10 (e.g. 10, 100, 1000 or more) different compounds.

A library of compounds of formula I wherein R^1 represents a small and hydrophobic group e.g. C_{2-4}alkyl or C_{2-4}alkenyl, especially propyl or isopropyl, particularly isopropyl may be particularly useful for screening for an inhibitor of elastase-like enzymes e.g. neutrophil elastase.

A library of compounds of formula I wherein R^1 represents a basic group e.g. $(CH_2)_{2-4}NHC(=NH)NH_2$, $(CH_2)_{1-2}PhC(=NH)NH_2$, $(CH_2)_{3-5}C(=NH)NH_2$, CH_2(cyclohexyl)NH_2, $(CH_2)_{1-3}(NH)_{0-1}$Het (wherein Het represents a 5 or 6 membered aromatic ring containing 1 or more nitrogen atoms and optionally substituted by amine) or $(CH_2)_{3-5}NH_2$ especially $(CH_2)_4C(=NH)NH_2$ or $(CH_2)_3NHC(=NH)NH_2$, particularly $(CH_2)_4C(=NH)NH_2$ may be especially useful for screening for an inhibitor of a trypsin-like enzyme (e.g. thrombin or tryptase).

A library of compounds of formula I wherein R^1 represents a large and hydrophobic group e.g. $(CH_2)_{1-2}Ph$, $(CH_2)_{0-2}$cyclohexyl or t-butyl may be useful for screening for an inhibitor of a chymotrypsin-like enzyme e.g.chymotrypsin or cathepsin G.

Library technology will be known to a person skilled in the art and is reviewed in Drug Discovery Today (1996) 1(4) 134-144 and Annual Reports in Combinatorial Chemistry and Molecular Diversity 1. Ed. Moos Walter H, Pavia Michael R, Kay Brian K, Ellington Andy D.

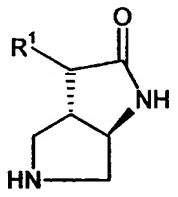
The library may be a solid phase or a solution phase library. It may be a discrete library or a pooled library.

We also provide a method of treatment of a disease in which serine protease activity is implicated which comprises administering to a patient an effective amount of compound of the invention; and use of a compound of the invention in the manufacture of a medicament for the treatment of a disease in which serine protease activity is implicated.

It will be appreciated that references herein to treatment extend to prophylaxis as well as the treatment of established conditions.

A particularly preferred embodiment of the invention relates to the application of compounds of the invention in the inhibition of neutrophil elastase, thrombin, and tryptase.

Compounds of the invention may be prepared from compounds of formula II

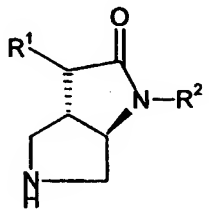


(II)

(relative stereochemistry indicated)

wherein R^1 is a moiety adapted to fit in the S_1 specificity subsite of the enzyme; or a protected derivative thereof, by sequential reaction to introduce the desired R^2 and R^3 substituent.

The following compounds are also useful intermediates.



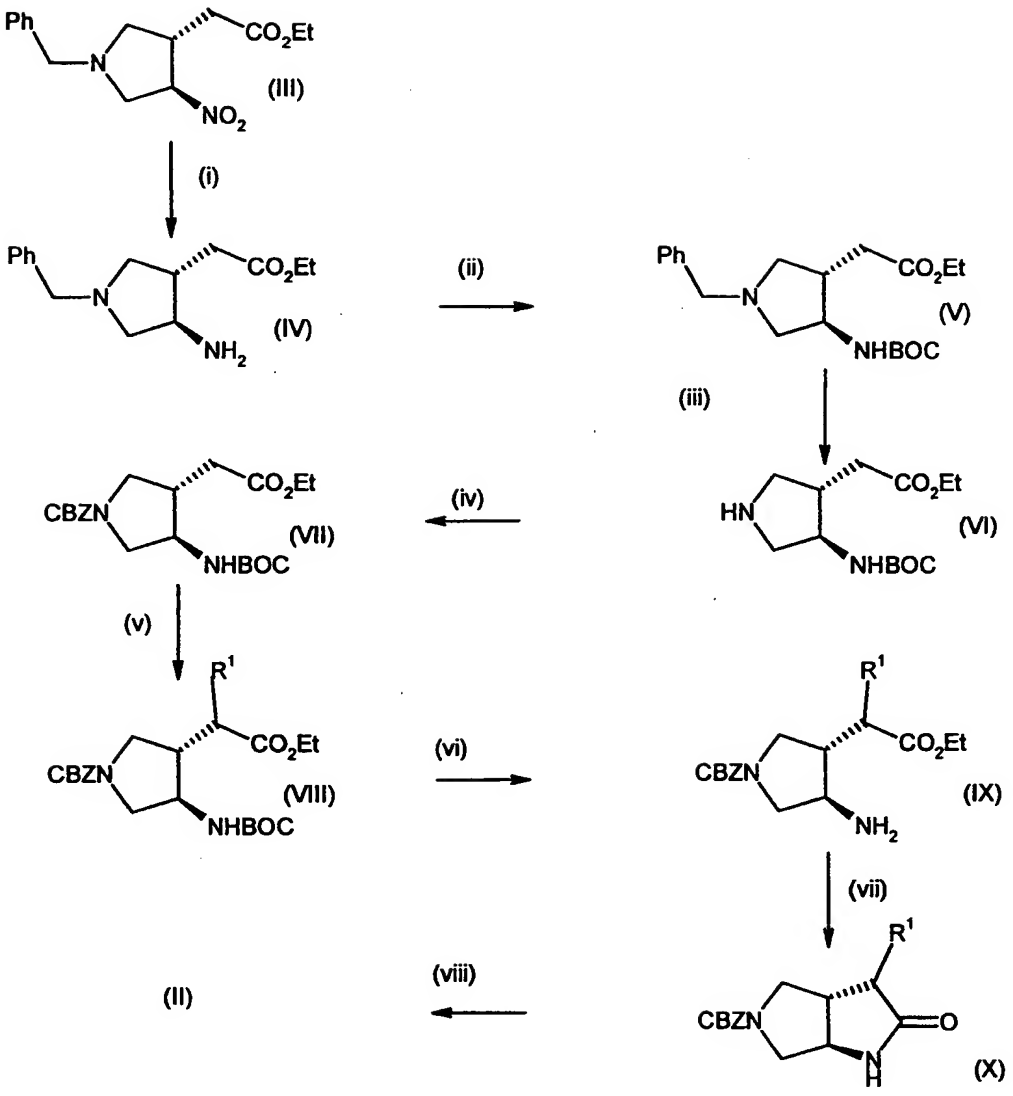
(II)'

Conditions for such sequential reactions will be known to a person skilled in the art. Generally these reactions will consist of alkylations (usually with an alkyl halide), sulphonylations (with a sulphonyl halide), acylations (reaction with a carboxylic acid, acid halide or anhydride) or conversion into a urea (eg by

reaction with an isocyanate) or conversion into a carbamate (eg by reaction with a haloformate ester). As an illustrative example, when R^2 represents $-SO_2Me$, the compound of formula II (or a protected derivative thereof) may be reacted with $MeSO_2Cl$.

Compounds of formula II may be prepared following Scheme 1 below (compounds are drawn with relative stereochemistry):

Scheme 1


Ph
N
CO2Et
NO2
(III)
(i)
NH2
(IV)
(ii)
(V)
NHBOC
(iii)
HN
(VI)
(iv)
CBZN
(VII)
(v)
R1
(VIII)
(vi)
(IX)
(vii)
(viii)
(II)
O
(X)

Step i

This is a standard nitro group reduction which may be achieved using hydrogen in the presence of a metal catalyst, e.g. palladium supported on carbon, in a suitable solvent such as ethanol.

The compound of formula III may be prepared by the method of Archille Barco *et al* (1992), J. Org. Chem., 57, 6279.

Step ii

This is a conventional protection reaction which may be performed by reacting with BOC_2O in the presence of a base, e.g. triethylamine, in an inert solvent such as acetonitrile or dichloromethane.

Step iii

This is a conventional deprotection reaction which may be achieved by reacting with hydrogen or ammonium formate in the presence of a metal catalyst, e.g. palladium supported on carbon, in a suitable solvent such as ethanol.

Step iv

This is a conventional protection reaction which may be performed by reacting with benzyl chloroformate in the presence of a base, e.g. triethylamine, in an inert solvent such as acetonitrile or dichloromethane.

Step v

This is generally an alkylation reaction which may be achieved by the sequential reactions with a strong base, e.g. LHMDS, in the presence of DMPU in an inert solvent such as THF or diethyl ether at -70°C, followed by alkyl halide, e.g. bromide or iodide, at -70°C -room temperature. Other R^1 side chains may be introduced by conventional processes.

Judicious choice of conditions may enable the preferred diastereomer to be produced preferentially.

Step vi

This is a conventional deprotection reaction which may be performed by reacting with an acid, e.g. trifluoroacetic acid or hydrogen chloride in a suitable solvent such as dichloromethane or dioxane.

Step vii

This cyclisation may be achieved using t-butyl magnesium chloride in the presence of TMEDA in an inert solvent such as THF or diethyl ether. The use of TMEDA is optional.

Alternatively this cyclisation may be performed by first hydrolysing the carboxylic acid ester (e.g. with sodium hydroxide) and then cyclising the resultant acid using diphenylphosphoryl azide in the presence of triethylamine in an inert solvent such as DMF or THF.

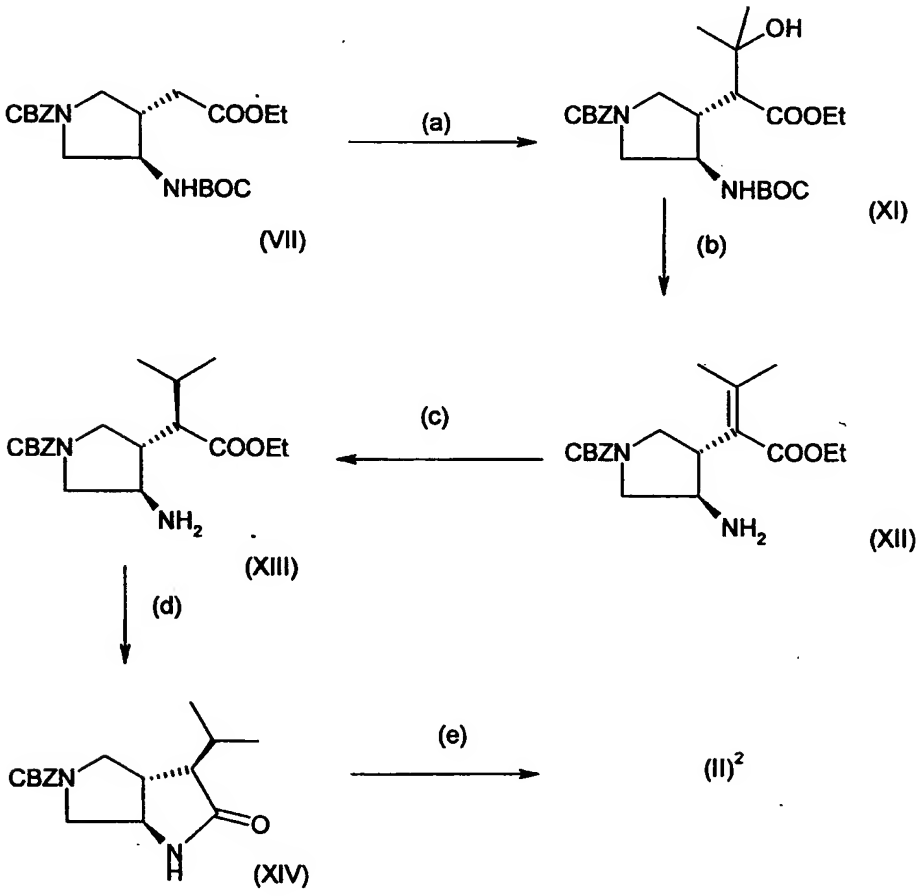
Step viii

This conventional deprotection reaction can be achieved by reacting with hydrogen in the presence of a metal catalyst, e.g. palladium supported on

carbon, and a proton source, e.g. ethereal hydrogen chloride, in a suitable solvent such as ethyl acetate or ethanol. The use of a proton source is optional.

Certain compounds of formula (II) in which R^1 represents isopropyl may alternatively be prepared by following an alternative Scheme 2 from compounds of formula (VII) (compounds are drawn with relative stereochemistry):

Scheme 2



Step (a)

For this reaction, the anion of the compound of formula (VII) is first prepared by treatment with a strong base, e.g. LHMDS, followed by treatment with acetone.

Step (b)

Standard dehydration conditions may be used for this reaction, e.g. treatment with concentrated sulphuric acid. These conditions will remove the BOC deprotecting group, otherwise the deprotection can be performed as an additional step.

Step (c)

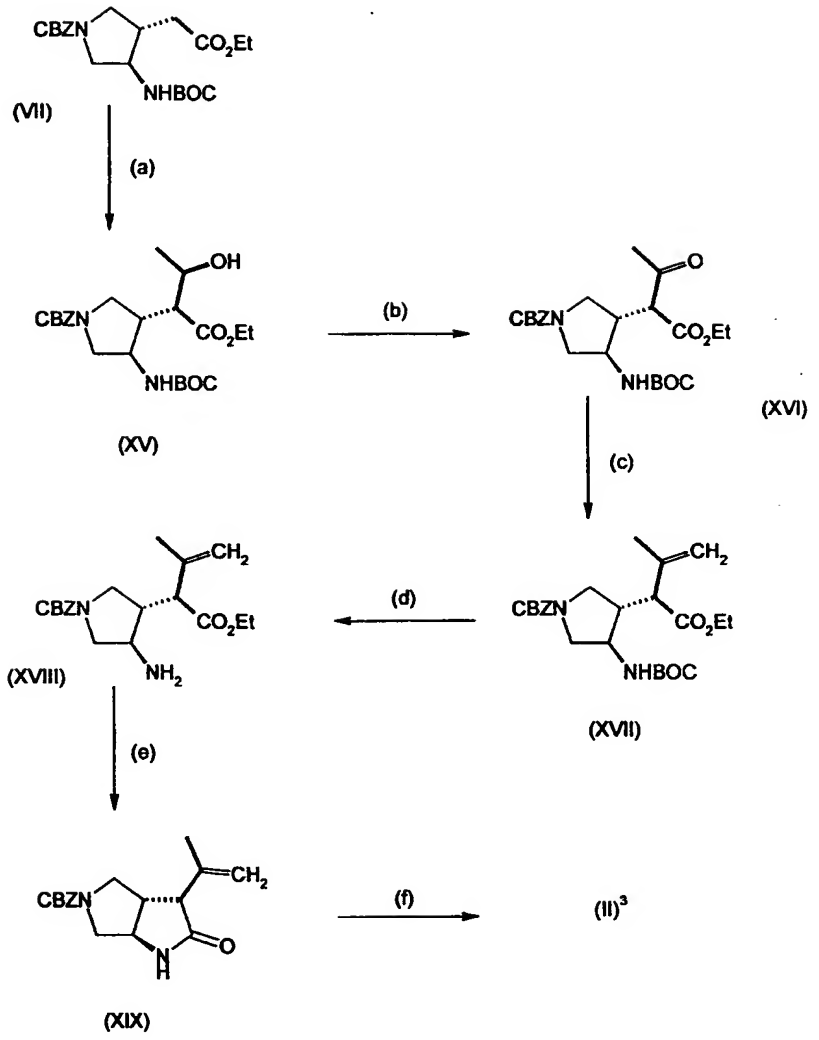
This is a reduction reaction which will be performed under mild conditions so as not to remove the CBZ protecting group. Hydrogenation over Pd/C in ethanol or ethyl acetate for a short period of time (eg <1hr) will be suitable to preferentially give the diastereoisomer illustrated.

Steps (d)-(e)

These processes are analogous to Scheme I, steps (vii)-(viii).

A further alternative method for preparation of compounds of formula (II) in which R^1 represents isopropyl is given in Scheme 3 (compounds are drawn with relative stereochemistry):

Scheme 3



Step (a)

For this reaction, the anion of compound of formula (VII) is prepared by treatment with a strong base, e.g. LHMDS, followed by treatment with acetaldehyde.

Step (b)

This oxidation may be suitably carried out under nitrogen using Swern oxidation conditions, e.g. oxalyl chloride and DMSO at -80°-0° in dichloromethane, followed by triethylamine.

Step (c)

This methylenation may be achieved using Tebbe reaction conditions, e.g. μ-chlorobis(η^5-2,4-cyclopentadien-1-yl)(dimethylaluminium)-μ-methylenetitanium in THF at around 0°- room temperature.

Step (d)

This standard deprotection may be performed using an acid, e.g. trifluroacetic acid or hydorogen chloride , in a suitable solvent such as dichloromethane or dioxane.

Step (e)

This cyclisation may be achieved using t-butyl magnesium chloride in an inert solvent such as THF or diethyl ether.

Step (f)

This process is analogous to Scheme 1, step (viii).

In Schemes 1, 2 and 3, a diastereomeric separation may be necessary to obtain the compound of desired stereochemistry.

The protecting groups CBZ and BOC used in Schemes 1, 2 and 3 are preferred illustrations and alternative protecting groups can be contemplated.

The route shown in Scheme 2 may also be followed for the preparation of compounds having different R^1, especially bulky R^1. A different ketone reagent will then be used in step (a).

Scheme 3 may also be adapted to produce compounds of formula (II) having other branched R^2 alkyl or alkenyl sidechains.

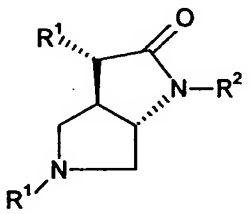
Compounds of formula (I) may also be prepared from another compound of formula (I) following one or more conventional chemical transformations.

It will be apparent to a person skilled in the art that the above synthetic processes for the preparation of compounds of formula (I) may be modified so as to include or omit protecting groups or so as to use alternative protecting groups (for example those described in T W Greene "Protective Groups Inorganic Synthesis", 2nd Ed (1991) J Wiley & Sons) in the course of routine optimisation of experimental conditions.

For example, when R^1 contains an amidine moiety, it may be preferred to introduce substituent R^1 (e.g. as in Scheme 1) as the oxadiazolinone derivative. This may be suitably O or N protected in subsequent chemical processes. Treatment of this derivative with hydrogen over Pd/C yields the free amidine.

The invention embraces compounds of the invention in racemic form as well as in a form in which one enantiomer predominates or is present exclusively. Generally, we prefer to provide a compound of formula (I) in diastereoisomerically and enantiomerically pure form.

For inhibition of elastase we prefer the diastereoisomers having relative stereochemistry shown in formula (1a)



(Ia)

Enantiomers having the absolute stereochemistry shown in formula (Ia) are especially preferred.

For inhibition of thrombin and tryptase we also prefer the diastereoisomers having relative stereochemistry shown in formula (Ia).

Enantiomerically pure compounds may be prepared by chiral separation or by synthesis based on chiral starting materials.

The present invention also covers the physiologically acceptable salts of the compounds of the invention. Suitable physiologically acceptable salts include inorganic base salts such as alkali metal salts (for example sodium and potassium salts) and ammonium salts and organic base salts. Suitable organic base salts include amine salts such as trialkylamine (e.g. triethylamine), dialkylamine (e.g. dicyclohexylamine), optionally substituted benzylamine (e.g. phenylbenzylamine or p-bromobenzylamine), procaine, ethanolamine,

diethanolamine, N-methylglucosamine and tri(hydroxymethyl)methylamine salts and amino acid salts (e.g. lysine and arginine salts). Suitable inorganic and organic acid salts include the hydrochloride, trifluoroacetate and tartrate.

The compounds of the invention may be formulated for administration in any convenient way, and the invention therefore also includes within its scope pharmaceutical compositions for use in therapy, comprising a compound of the invention or a physiologically acceptable salt or solvate thereof in admixture with one or more physiologically acceptable diluents or carriers.

There is also provided according to the invention a process for preparation of such a pharmaceutical composition which comprises mixing the ingredients as considered appropriate for the indication.

The compounds of the invention may, for example, be formulated for oral, buccal, parenteral, topical or rectal administration.

Tablets and capsules for oral administration may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, mucilage of starch or polyvinyl pyrrolidone; fillers, for example, lactose, microcrystalline cellulose, sugar, maize- starch, calcium phosphate or sorbitol; lubricants, for example, magnesium stearate, stearic acid, talc, polyethylene glycol or silica; disintegrants, for example, potato starch, croscarmellose sodium or sodium starch glycollate; or wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as

suspending agents, for example, sorbitol syrup, methyl cellulose, glucose/sugar syrup, gelatin, hydroxymethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example, lecithin, sorbitan mono-oleate or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters, propylene glycol or ethyl alcohol; or preservatives, for example, methyl or propyl p- hydroxybenzoates or sorbic acid. The preparations may also contain buffer salts, flavouring, colouring and/or sweetening agents (e.g. mannitol) as appropriate.

For buccal administration the compositions may take the form of tablets or lozenges formulated in conventional manner.

The compounds may also be formulated as suppositories, e.g. containing conventional suppository bases such as cocoa butter or other glycerides.

The compounds of the invention may also be formulated for parenteral administration by bolus injection or continuous infusion and may be presented in unit dose form, for instance as ampoules, vials, small volume infusions or pre-filled syringes, or in multi-dose containers with an added preservative. The compositions may take such forms as solutions, suspensions, or emulsions in aqueous or non-aqueous vehicles, and may contain formulatory agents such as anti-oxidants, buffers, antimicrobial agents and/or toxicity adjusting agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use. The dry solid presentation may be prepared by filling a sterile powder aseptically into individual sterile containers or by filling a sterile solution aseptically into each container and freeze-drying.

By topical administration as used herein, we include administration by insufflation and inhalation. Examples of various types of preparation for topical administration include ointments, creams, lotions, powders, pessaries, sprays, aerosols, capsules or cartridges for use in an inhaler or insufflator or drops (e.g. eye or nose drops).

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents and/or solvents. Such bases may thus, for example, include water and/or an oil such as liquid paraffin or a vegetable oil such as arachis oil or castor oil or a solvent such as a polyethylene glycol. Thickening agents which may be used include soft paraffin, aluminium stearate, cetostearyl alcohol, polyethylene glycols, microcrystalline wax and beeswax.

Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents or thickening agents.

Powders for external application may be formed with the aid of any suitable powder base, for example, talc, lactose or starch. Drops may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilising agents or suspending agents.

Spray compositions may be formulated, for example, as aqueous solutions or suspensions or as aerosols delivered from pressurised packs, with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, 1,1,1,2,3,3,3-heptafluoropropane, 1,1,1,2-tetrafluorethane, carbon dioxide or other suitable gas.

Capsules and cartridges for use in an inhaler or insufflator, of for example gelatin, may be formulated containing a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

Compounds of the invention may also be used in purification and diagnostic applications involving serine protease enzymes. For example, an immobilised compound of the invention may allow a serine protease capable of binding that compound to be isolated. A tagged compound of the invention may enable a serine protease capable of binding that compound to be identified.

ABBREVIATIONS

BOC	t-butyloxycarbonyl
CBZ	Benzyloxycarbonyl
$(BOC)_20$	Di-tert-butyldicarbonate
THF	Tetrahydrofuran
LHMDS	Lithium bis (trimethylsilyl)amide
DMPU	1,3-dimethyl-3,4,5,6-tetrahydro 2 (1H)-pyrimidinone
DMAP	4-dimethylaminopyridine
DMF	Dimethylformamide
EDC	1-(3-N,N-dimethylaminopropyl)-3-ethylcarbodiimide
DEAD	diethylazodicarboxylate
DCM	dichloromethane
TMEDA	tetramethylethylenediamine
DMSO	dimethylsulphoxide

The invention will be illustrated by reference to the following examples:

Assay Examples

In the foregoing, enzyme activity is generally determined at a 15 minute timepoint. Enzyme kinetics may be investigated by determining enzyme activity at other timepoints (e.g. 0, 30 minutes).

Assay Example 1

In vitro assay for inhibition of human neutrophil elastase

Assay contents:
50mM Tris/HCl (pH 8.6)
150mM NaCl
11.8nM purified human neutrophil elastase
Suitable concentrations of compound under test diluted with water from a 10mM stock solution in dimethylsulphoxide. Values above are final concentrations after the addition of substrate solution (see below).

The mixture above is incubated for 15 minutes at 30°C at which time the remaining elastase activity is measured for 10 minutes in a BioTek 340i plate-reader, after the addition of 0.6mM MeO-succinyl-Ala-Ala-Pro-Val-p-nitroanilide. The rate of increase in absorbance at 405nm is proportional to elastase activity. Enzyme activity is plotted against concentration of inhibitor and an IC_{50} determined using curve fitting software.

Assay Example 2
In vitro assay for inhibition of human thrombin

Compounds of the invention may be tested for their thrombin inhibitory activity as determined in vitro by their ability to inhibit human α-thrombin in a chromogenic assay, using N-p-tosyl-Gly-Pro-Lys p-nitroanilide as the chromogenic substrate. All dilutions were made in a buffer consisting of: 50mM HEPES, 150 mM NaCl, 5mM $CaCl_2$, 0.1% PEG and at pH7.4. Briefly, the substrate (final conc. of 100μM) was added to thrombin (final conc. of 1nM) and the reaction monitored for 10mins at 405nm using a Biotek EL340 plate reader; the assay was performed at room temperature. To obtain IC_{50} values the data were analysed using Kineticalc® with a 4-parameter curve fitting procedure to obtain the IC_{50} value. To determine the IC_{50} at 15mins, the compounds were

preincubated with thrombin for these times prior to adding the chromogenic substrate.

Assay Example 3

In vitro pNA assay of viral serine protease inhibitor activity

The hCMV serine protease used is a mutant of the 30K protease lacking the internal cleavage site (Ala142/Ala143) and which has been cloned in E.coli to produce active enzyme (hCMV δAla protease). IC_{50} data for test compounds are determined after preincubation of the enzyme with test inhibitor compound for 15 minutes. Test compounds are dissolved in DMSO, serially diluted and added at a range of concentrations (from 100μM - 0.195μM) to a reaction containing 0.5μM CMV δAla protease, 100mM HEPES pH7.5, 0.2mM EDTA, 10mM NaCl, 1mM DTT, and 30% glycerol. The reaction mixture is pre-incubated at 32°C for 15 minutes prior to addition of 4mM oligopeptide substrate (Arg-Glu-Ser-Tyr-Val-Lys-Ala-pNA) and then analysed at 32°C in a BIO-TEK Bio Kinetics Reader EL340i. The plate reader monitors production of pNA and calculates the reaction rates over 30 minutes. The rates are plotted against inhibitor concentration and IC_{50} values determined.

Assay Example 4

In vitro assay for inhibition of human mast cell tryptase.

Compounds of the invention may be tested for their tryptase inhibitory activity as determined in vitro by their ability to inhibit human lung mast cell tryptase in a chromogenic assay, using N-p-Tosyl-Gly-Pro-Lys-p-nitroanilide as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of: 10mM Tris-HCl, 120mM NaCl, pH 7.4. Briefly, the substrate (final conc. of 400μM) was

added to tryptase (final conc. of 0.11μg.ml^{-1}) and compound at appropriate concentrations and the reaction monitored for 30 minutes at 405nm using a Molecular Devices Thermomax microplate reader; the assay was performed at room temperature. To obtain IC_{50} values the data were analyzed using curve fitting software. To determine the IC_{50} at 30 mins. the compounds were preincubated with tryptase for this time prior to addition of the chromogenic substrate.

Assay Example 5

In vitro assay for inhibition of trypsin

Compounds of the invention may be tested for their trypsin inhibitory activity as determined in vitro by their ability to inhibit bovine trypsin in a chromogenic assay, using N-Benzoyl-Ile-Glu-Gly-Arg-p-nitroanilide as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of: 50mM Tris-HCl, 15mM $CaCl_2$, pH 8.4. Briefly, the substrate (final conc. of 160μM) was added to trypsin (final conc. of 25ng.ml^{-1}) and compound at appropriate concentrations incubated for 15 minutes and the reaction monitored for 10 minutes at 405nm using a BioTek EL340 plate reader; the assay was performed at 37°C. To obtain IC_{50} values the data were analyzed using Kineticalc® with a 4-parameter curve-fitting procedure.

Assay Example 6

In vitro assay for inhibition of Factor Xa

Compounds of the invention may be tested for their Factor Xa inhibitory activity as determined in vitro by their ability to inhibit human Factor Xa in a chromogenic assay, using N-α-Benzyloxycarbonyl-D-Arg-Gly-Arg-p-nitroanilide

as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of: 50mM Tris-HCl, 150mM NaCl, 5mM $CaCl_2$, pH 7.4. Briefly, the substrate (final conc. of 200μM) was added to Factor Xa (final conc. of 0.02 $U.ml^{-1}$) and compound at appropriate concentrations incubated for 15 minutes and the reaction monitored for 10 minutes at 405nm using a BioTek EL340 plate reader; the assay was performed at 37°C. To obtain IC_{50} values the data were analyzed using Kineticalc® with a 4-parameter curve fitting procedure.

Assay Example 7

In vitro assay for inhibition of Factor XIa

Compounds of the invention may be tested for their Factor XIa inhibitory activity as determined in vitro by their ability to inhibit human Factor XIa in a chromogenic assay, using L-Pyroglutamyl-Pro-Arg-p-nitroanilide as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of: 8.1mM NaH_2PO_4, 147mM KH_2PO_4, 2.7mM KCl, 137mM NaCl, pH 7.2. Briefly, the substrate (final conc. of 400μM) was added to Factor XIa (final conc. of 0.25$\mu g.ml^{-1}$) and compound at appropriate concentrations incubated for 15 minutes and the reaction monitored for 10 minutes at 405nm using a BioTek EL340 plate reader; the assay was performed at 25°C. To obtain IC_{50} values the data were analyzed using Kineticalc® with a 4-parameter curve fitting procedure.

Assay Example 8

In vitro assay for inhibition of Factor XIIa

Compounds of the invention may be tested for their Factor XIIa inhibitory activity as determined in vitro by their ability to inhibit human Factor XIIa in a

chromogenic assay, using H-D-Pro-Phe-Arg-p-nitroanilide as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of: 28mM NaBarbitone, 125mM NaCl, 1mM EDTA, pH 7.35. Briefly, the substrate (final conc. of 200μM) was added to Factor XIIa (final conc. of 1.25μg.ml^{-1}) and compound at appropriate concentrations incubated for 15 minutes and the reaction monitored for 10 minutes at 405nm using a BioTek EL340 plate reader; the assay was performed at 25°C.

Assay Example 9

In vitro assay for inhibition of tPA

Compounds of the invention may be tested for their tissue plasminogen activator inhibitory activity as determined in vitro by their ability to inhibit human tissue plasminogen activator in a chromogenic assay, using $MeSO_2$-D-CHT-Gly-Arg-p-nitroanilide as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of: 50mM Tris-HCl, 150mM NaCl, pH 8.4. Briefly, the substrate (final conc. of 750μM) was added to tissue plasminogen activator (final conc. of 1.0μg.ml^{-1}) and compound at appropriate concentrations incubated for 15 minutes and the reaction monitored for 10 minutes at 405nm using a BioTek EL340 plate reader; the assay was performed at 30°C. To obtain IC_{50} values the data were analyzed using Kineticalc® with a 4-parameter curve-fitting procedure.

Assay Example 10

In vitro assay for inhibition of plasmin

Compounds of the invention may be tested for their plasmin inhibitory activity as determined in vitro by their ability to inhibit human plasmin in a chromogenic

assay, using H-D-Val-Leu-Lys-p-nitroanilide as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of: 50mM Tris-HCl, 150mM NaCl, 5mM $CaCl_2$, pH 7.4. Briefly, the substrate (final conc. of 363μM) was added to plasmin (final conc. of 0.02 $U.ml^{-1}$) and compound at appropriate concentrations incubated for 15 minutes and the reaction monitored for 10 minutes at 405nm using a BioTek EL340 plate reader; the assay was performed at 37°C. To obtain IC_{50} values the data were analyzed using Kineticalc® with a 4-parameter curve fitting procedure.

Assay Example 11

In vitro assay for inhibition of Factor VIIa

Compounds of the invention may be tested for their Factor VIIa inhibitory activity as determined in vitro by their ability to inhibit human Factor VIIa in a chromogenic assay, using H-D-Ile-Pro-Arg-p-nitroanilide as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of: 20mM Tris-HCl, 150mM NaCl, 5mM $CaCl_2$, 0.1% bovine serum albumin, pH 7.5. Briefly, the substrate (final conc. of 400μM) was added to Factor VIIa (final conc. of 10nM in the presence of recombinant soluble tissue factor at optimal concentration) and compound at appropriate concentrations incubated for 15 minutes and the reaction monitored for 30 minutes at 405nm using a BioTek EL340 plate reader; the assay was performed at 37°C. To obtain IC_{50} values the data were analyzed using. Kineticalc® with a 4-parameter curve fitting procedure.

Assay Example 12

In vitro assay for inhibition of chymotrypsin

Compounds of the invention may be tested for their chymotrypsin inhibitory activity as determined in-vitro by their ability to inhibit human pancreatic chymotrypsin in a chromogenic assay, using MeO-Succ-Arg-Pro-Tyr-pNA hydrochloride as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of 50mM Tris-HCl, 150mM NaCl, 25mM $CaCl_2$, pH 8.4. Briefly, the substrate (final conc. of 178μM) was added to chymotrypsin (final conc. of 0.2μg/mL) and compound at appropriate concentrations and the reaction monitored for 10 minutes at 405nm using a BioTek EL340 plate reader: the assay was performed at 30°C. To obtain IC_{50} values the data were analysed using Kineticalc® with a 4-parameter curve fitting procedure. To determine the IC_{50} at 15 mins. the compounds were preincubated with chymotrypsin for these times prior to addition of the chromogenic substrate.

Assay Example 13

In vitro assay for inhibition of cathepsin G

Compounds of the invention may be tested for their Cathepsin G inhibitory activity as determined *in vitro* by their ability to inhibit human neutrophil Cathepsin G in a chromogenic assay, using N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide as the chromogenic substrate. Compounds were diluted from a 10mM stock solution in dimethylsulphoxide. All dilutions were made in a buffer consisting of: 100mM HEPES, 300mM NaCl, pH 7.2. Briefly, the enzyme (1.25ug/mL final), buffer and compound at appropriate concentrations were incubated for 15 mins at 30°C. Substrate (7.25mM final) was added and the reaction monitored at 30°C for 30 minutes at 405nm using a BioTek EL340 plate reader. To obtain IC_{50} values the data were analyzed using Microsoft Excel® within ActivityBase® with a 4-parameter curve fitting procedure (XLFIT®).

Assay Example 14

In vitro assay for plasma stability

Stability of the compounds of the invention to exposure to undefined esterolytic (and other) activity was assessed in rat plasma and blood. Briefly, compounds were mixed with fresh rat plasma or rat blood, then incubated at 37°C and at various times after mixing were extracted by precipitation with acetonitrile. Reverse phase high performance liquid chromatography was used to quantify compounds at each time point. Half-lives for the compounds were calculated from the time-course data by log-linear regression. Compounds were considered to be unstable if the half life was less than 10 minutes.

Assay Example 15

Tests for acylating enzyme inhibitors

The mechanism of binding of the compounds was determined using biophysical techniques such as mass spectrometry and X-ray crystallography. Briefly, crystal structures were prepared in complex with thrombin by soaking and co-crystallisation. X-ray data was collected using a FAST area detector system and difference fourier analysis identified the binding modes of the inhibitors. The bound conformations for each inhibitor were obtained after subsequent refinement cycles which often identified an acylation event.

Active thrombin, after incubation with the compounds, was examined by liquid chromatography coupled in-line to a mass spectrometer. If the measured mass of the thrombin bearing the active site serine residue was increased by the mass of the inhibitor this indicated the formation of a covalent bond between the two.

Compound Examples

Compound Example 1

rel-(3R,3aS,6aR)-1-Methanesulfonyl-5-(4-piperidin-1-yl-but-2-enoyl)-3-propyl-hexahydro-pyrrolo[3,4-b]pyrrol-2-one hydrochloride

(a) rel-(3S,4R)-(1-Benzyl-4-nitro-pyrrolidin-3-yl)-acetic acid ethyl ester

This compound was prepared by the method of Archille Barco *et al* J.Org.Chem.1992, *57*, 6279 as a medium brown oil. Tlc (cyclohexane:ethyl acetate:3:1) Rf 0.26.

(b) rel-(3S,4R)-(4-Amino-1-benzyl-pyrrolidin-3-yl)-acetic acid ethyl ester

A solution of the product of step (a) (397g) in absolute ethanol (1.4l) was reduced at room temperature with stirring over a platinum/carbon catalyst (5% 115g) under a hydrogen atmosphere. The reaction proceeded to completion over 5h (H_2 uptake 99.7l). The reaction mixture was filtered through celite and evaporated *in vacuo* to give the title compound as a light brown oil (303g). Tlc Silica, cyclohexane:ethyl acetate (3:1); Rf 0.15

(c) rel-(3S,4R)-(1-Benzyl-4-tert-butoxycarbonylamino-pyrrolidin-3-yl)-acetic acid ethyl ester

A solution of (289g) in acetonitrile (1.5l) was added below 25°C with stirring over 25 min to a solution of the product of step (b) (288.8g) and triethylamine (111.2g) in acetonitrile (1.5l). After 2.5 days the solvent was removed *in vacuo* and the resultant brown oil partitioned between ethyl acetate (2.5l) and water (2l). The aqueous layer was further extracted with ethyl acetate (2l). The combined organics were washed with brine, dried ($MgSO_4$), filtered and evaporated. The residual light brown solid was triturated with ether and then

collected by filtration. The title compound was obtained as a white solid (233g). Tlc Silica, hexane:ethylacetate (3:1); Rf 0.27

(d) rel-(3S,4R)-(4-tert-Butoxycarbonylamino-pyrrolidin-3-yl)-acetic acid ethyl ester

A solution of the product of step (c) (100g) in absolute ethanol (1.25l) was reduced over palladium on carbon catalyst (10%, 20g) under a hydrogen atmosphere at room temperature and with stirring (Hydrogen uptake 7.5l). After 20h the reaction mixture was filtered through hyflo and evaporated *in vacuo* to give the title compound as a colourless oil (71g) which crystallised to a white solid on standing. Tlc (dichloromethane; ethanol; ammonia 80:20:2) Rf 0.4.

(e) rel-(3S,4R)-3-tert-Butoxycarbonylamino-4-ethoxycarbonylmethyl-pyrrolidine-1-carboxylic acid benzyl ester

Triethylamine (156ml) was added slowly to a cooled solution of the product of step (d) (100.7g) and benzyl chloroformate (74ml) in dichloromethane (3l) at 10°C with stirring. The temperature during the addition was maintained below 15°C. The reaction mixture was stirred overnight and then washed with water (3l). The solvent was removed *in vacuo* and the residual oil purified by chromatography on silica (Merck 9385) eluting with hexane:ethyl acetate (3:1). The resultant white solid was triturated with hexane:ethyl acetate (2:1) (400ml), to give the title compound 155g. Mass spec. (found) MH^+ 407 (calc) MH^+ 407.

(f) rel-(3S,4R)-3-tert-Butoxycarbonylamino-4-(1-ethoxycarbonyl-but-3-enyl)-pyrrolidine-1-carboxylic acid benzyl ester

A solution of lithium bis (trimethylsilyl) amide in tetrahydrofuran (1M, 80ml) was added dropwise over 1h with stirring under a nitrogen atmosphere at -70°C to a solution of the product of step (e) (9.8g) in a mixture of dry tetrahydrofuran

(54ml) and 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H) pyrimidinone (120ml). After a further 1h allyl iodide (2.8ml) was added dropwise over 10 min and the mixture was stirred below -70°C for 2h. The reaction was quenched with saturated aqueous ammonium chloride (30ml) which was added dropwise over 30 min. The mixture was extracted with ethyl acetate (4x100ml). The combined extracts were dried ($MgSO_4$), filtered and evaporated *in vacuo* to give an orange oil (26g). The oil was partitioned between toluene (200ml) and water (100ml). The organics were washed with water (2x100ml) and brine (80ml), dried (Na_2SO_4), filtered and evaporated to give a clear orange oil (15g). Purification of this oil by flash column chromatography on silica (Merck 9385) using gradient elution [ethyl acetate:hexane from (1:9) to (2:3)]. The title compound was thereby obtained as a white crystalline solid (8.6g). Mass spec MH^+ (found) 447 MH^+ (calc) 447

(g) rel-(3R,4S)-3-Amino-4-(1-ethoxycarbonyl-but-3-enyl)-pyrrolidine-1-carboxylic acid benzyl ester

The product of step (f) (5g) was dissolved in 4M hydrogen chloride in dioxan (53ml) at room temperature. The solution was stirred for 3h and evaporated. The residue was partitioned between water (100ml) and ether (50ml). The aqueous layer was washed with ether (50ml) and then basified with saturated aqueous sodium hydrogen carbonate (100ml). The mixture was extracted with ethyl acetate (3x50ml). The combined extracts were dried (Na_2SO_4) filtered and evaporated to give the title compound as a clear, pale yellow, viscous oil (3.7g). Mass spec MH^+ (found) 347 MH^+ (calc) 347.

(h) rel-(3R,3aS,6aR)-3-Allyl-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-5-carboxylic acid benzyl ester

A solution of the product of step (g) (3.5g) in a mixture of dry tetrahydrofuran (35ml) and dry tetramethylethylenidiamine (35ml) was cooled to 5°C. A solution of t-butyl magnesium chloride (1M, 32ml) in tetrahydrofuran was added over 25 min and the mixture was allowed to warm to room temperature over 1.5h. After a further 1.5h the reaction was quenched with saturated aqueous ammonium chloride (10ml). Chilled 2M hydrochloric acid (100ml) was then added with cooling. The resultant mixture was acidified (pH1-2) using concentrated hydrochloric acid (40ml). The mixture was extracted with ethyl acetate (3x100ml). The combined extracts were washed with brine, dried (Na_2SO_4), filtered and evaporated to give the title compound as a white crystalline solid (3g). Mass spec MH^+ (found) 301 MH^+ (calc) 301.

(i) rel-(3R,3aS,6aR)-3-allyl-methanesulfonyl-2-oxo-hexahydropyrrolo[3,4-b]pyrrole-5-carboxylic acid benzyl ester

A solution of lithium hexamethyldisilazide in tetrahydrofuran (1M, 13ml) was added at -10°C over 10 min to a stirred solution of the product of step (h) (3g). After 5 min the reaction was allowed to warm to around 0°C, at which temperature it was held for 35 min. The mixture was recooled to -70°C and methanesulphonyl chloride (1.93ml) was added dropwise over 15 min. After a further 3.5h the reaction was quenched with saturated aqueous ammonium carbonate (20ml) at below -50°C. It was then allowed to attain ambient temperature, when it was diluted with water (100ml) and extracted with ethyl acetate (3x100ml). The combined extracts were washed with brine (100ml), dried (Na_2SO_4), filtered and evaporated. The resultant oil was purified by flash column chromatography on silica using gradient elution. The eluent was initially ethyl acetate hexane (4:1) and increased in palarity to ethyl acetate:hexane (5:1). The title compound was obtained as a viscous gum/glass (3.1g). Mass spec MH^+ (found) 379 MH^+ (calc) 379

(j) rel-(3R,3aS,6aR)-1-Methanesufonyl-3-propyl-hexahydro-pyrrolo[3,4-b]pyrrol-2-one hydrochloride

A solution of the product of step (i) (3g) in ethyl acetate (150ml) containing ethereal hydrogen chloride (1.0M, 12ml) was stirred over 5% palladium on activated carbon (wet 1.5g) under a hydrogen atmosphere. After 5h more catalyst (0.5g) was added and the reaction was stirred for a further 16h. (Hydrogen uptake 470ml). The reaction mixture was diluted with an equal volume of absolute ethanol and then filtered through celite. The filtrate was evaporated, redissolved in a mixture of ethyl acetate and dichloromethane (1:10, 100ml) and extracted with water (2x50ml). The combined extracts were washed with ethyl acetate (50ml), basified with saturated aqueous sodium hydrogen carbonate and extracted with dichloromethane (4x50ml). The combined extracts were dried (Na_2SO_4) filtered and evaporated to give a pale yellow foam (1.2g). Ethereal hydrogen chloride (1.0M, 5ml) was added and the mixture reevaporated to give the title compound as a glass. T.l.c. (dichloromethane; ethanol: ammonia, 100:8:1) Rf 0.1.

(k) 4-(Piperidin-1-yl)-but-2-enoic Acid Anhydride Dihydrochloride

Oxalyl chloride (4.2ml) was added to a stirred solution of 4-(piperidin-1-yl)-but-2-enoic acid hydrochloride (10g) in dichloromethane (200ml). Dimethylformamide (2 drops) was then added. The resultant suspension was stirred for 4h and then concentrated to ~25ml. The slurry obtained was stirred a further 1h and then filtered. The filter pad was washed with DCM (50ml) and dried *in vacuo* to give the title compound as a white solid (4.95g). M.p. 120-124°C (decomposition).

(l) rel-(3R,3aS,6aR)-1-Methanesulfonyl-5-(4-piperidin-1-yl-but-2-enoyl)-3-propyl-hexahydro-pyrrolo[3,4-b]pyrrol-2-one hydrochloride

The product of step (k) (0.44g) was added at room temperature to a solution of the product of step (j) (0.28g) in DCM (20ml) containing solid sodium hydrogen carbonate (0.34g). The reaction was stirred for 4h. The mixture was washed with saturated aqueous sodium hydrogen carbonate (20ml). The aqueous phase was separated and extracted with dichloromethane (20ml). The combined organics were washed with water (2 x 30ml), dried over sodium sulphate and evaporated *in vacuo*. The resultant gum was purified by flash column chromatography on silica gel (Merck 9385) using gradient elution. The initial eluent, dichloromethane:ethanol:ammonia (200:8:1), was increased in polarity to dichloromethane:ethanol:ammonia (150:8:1). The resultant viscous oil (0.42g) was dissolved in ethyl acetate (30ml) and treated with ethereal hydrogen chloride. The resultant white solid suspension was evaporated and dried *in vacuo* to give the title compound. T.l.c. silica, dichloromethane: ethanol:ammonia (100:8:1); Rf. 0.55. Mass spec. MH^+ (found) 398, MH^+ (calc) 398.

Compound Example 2

rel-N-[4-(1-Methanesulfonyl-2-oxo-3R-propylhexahydro-(3aS,6aR)-pyrrolo[3,4-b]pyrrole-5-sulfonyl)-phenyl]acetamide

Triethylamine (246µl) and N-acetylsulfanilyl chloride (108mg) were added to a solution of the product of Compound Example 1, step (j) (100mg) in dichloromethane (3.3ml). The solution was stirred at room temperature for 5h. Sodium hydrogen carbonate (150mg) was added and the reaction stirred overnight. The resultant suspension was washed with saturated sodium hydrogen carbonate solution. The solvent was removed *in vacuo*. The resultant

solid was purified using pre-packed silica cartridges. The columns were eluted with vacuum suction using the following solvents; dichloromethane (2 x column volume), chloroform (2 x column volume), diethyl ether (4 x column volume), ethyl acetate (4 x column volume) and acetonitrile (4 x column volume) to give the title compound (48mg) as a white solid. T.l.c. silica, dichloromethane:methanol, 9:1; Rf. 0.61. Mass spec. MH^+ (found) 444, MH^+ (calc) 444.

Compound Example 3

rel-(3R,3aR,6aS)-3-(4-Carbamimidoyl-butyl)-5-(1H-indole-2-carbonyl)-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-1-carboxylic acid methyl ester trifluoro-acetate

(a) 3-(4-Iodo-butyl)-5-trichloromethyl-[1,2,4]oxadiazole

To a solution of 1-iodo-5-nitro-pentane (82g) in toluene (820ml) was added trichloroacetonitrile (34ml), triethylamine (3ml) and phenylisocyanate (74ml). The mixture was then placed in an ultrasound bath for 18h. Hexane was added to the reaction and the mixture filtered. The filtrate was concentrated under reduced pressure and the crude product was purified by flash column chromatography eluting with diethyl ether:hexane, (2:98) and (4:98), to give the title compound (110g) as an orange oil. T.l.c. (Silica, hexane:diethyl ether, 9:1) Rf 0.5

(b) 3-(4-Iodo-butyl)-4H-[1,2,4]oxadiazol-5-one

To a solution of the product of step (a) (85g) in ethanol (1500ml) was added potassium hydroxide (15.3g). The resultant mixture was stirred at room temperature for 3.5h and then concentrated under reduced pressure. The residue was then partitioned between sodium carbonate (2N) and diethyl ether. The aqueous layer was acidified to pH2 with concentrated hydrochloric acid and then extracted with diethyl ether. The combined organic extracts were dried (sodium sulfate) and concentrated under reduced pressure to give, after

triturating in diethyl ether, the title compound (37g) as a white solid. Analysis: Found: C,27.0; H,3.4; N,10.35%

$C_6H_9IN_2O_2$ requires C,26.9; H,3.4; N,10.45%

(c) trans-3-tert-Butoxycarbonylamino-4-[1-ethoxycarbonyl-5-(5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-yl)-pentyl]-pyrrolidine-1-carboxylic acid benzyl ester

A solution of the product of Compound Example 1, step (e) (5.2g) in THF (110ml) at -79°C was treated with a THF solution of lithium hexamethyldisilazide (1M, 60ml) over 20 min. After 2.5h at -76°C, the product of step (b) (4.18g) in THF/HMPA (27ml/35ml) was added over 20 min and the resultant solution was maintained at -70°C for 2.0h. After reaching 0°C (over 1h), the reaction was quenched with ammonium chloride solution and diluted with water. The resultant solution was extracted with ethyl acetate and the combined organic extracts were washed with water and brine, dried (magnesium sulfate) and concentrated under reduced pressure. The crude product was subjected to flash column chromatography eluting with cyclohexane:ethyl acetate:acetic acid (60:40:2) to give the title compound (4.4g) as a yellow oil. Analysis: Found: C,57.35; H,7.4; N,9.5%

$C_{27}H_{38}N_4O_8$ $1CH_3CO_2H$ requires C,57.3; H,6.9; N,9.2%

(d) trans-3-Amino-4-[1-ethoxycarbonyl-5-(5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-yl)-pentyl]-pyrrolidine-1-carboxylic acid benzyl ester trifluoro-acetate

The product of step (c) (4.3g) was dissolved in trifluoroacetic acid (20ml) and dichloromethane (175ml). After 2h, the solvents were removed under reduced pressure to give the title compound (4.0g) as a yellow oil.

Mass spectrum: Found: MH^+ 447

(e) trans-3-Amino-4-[1-carboxy-5-(5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-yl)-pentyl]-pyrrolidine-1-carboxylic acid benzyl ester hydrochloride

A mixture of the product of step (d) (4.4g), potassium carbonate (5.9g), ethanol (120ml) and water (120ml) was stirred at reflux under nitrogen for 3 days. The cooled mixture was concentrated under reduced pressure and the residue treated with hydrochloric acid (2M) and concentrated under reduced pressure. The residue was stirred in hot ethanol, filtered and the filtrate concentrated under reduced pressure to give the title compound (1.8g) as a brown foam.

Mass spectrum: Found: MH^+ 419

(f) rel-(3R,3aR,6aS)-2-Oxo-3-[4-(5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-yl)-butyl]-hexahydro-pyrrolo[3,4-b]pyrrole-5-carboxylic acid benzyl ester-GR216138X

A mixture of the product of step (e) (3.0g), diphenylphosphorylazide (2.8ml) and triethylamine (4.5ml) in DMF (150ml) was stirred at room temperature for 24h. The mixture was concentrated under reduced pressure and the residue subjected to preparative h.p.l.c. (gradient profile 30-80% (ii) in 15min). The title compound (1.52g) was obtained as a white solid by concentration of the required fraction under reduced pressure and drying by repetitive addition of acetonitrile and concentration under reduced pressure.

Analytical h.p.l.c. (gradient profile 10-90% (ii) in 25min) Rt 20.8min

(g) rel-(3R,3aR,6aS)-3-[4-(4-tert-Butoxycarbonyl-5-oxo-4,5-dihydro-[1,2,4] oxadiazol-3-yl)-butyl]-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-5-carboxylic acid benzyl ester

Triethylamine (0.781ml) was added to a stirred solution of the product of step (f) (0.75g) and di-tert-butylcarbonate (0.816g) in dry DMF (30ml) at room temperature. After 24h, the solution was poured into phosphate buffer (pH6.5) and extracted with ethyl acetate. The combined organic extracts were washed with water, brine, dried (sodium sulfate), and concentrated under reduced

pressure. The residue was subjected to preparative h.p.l.c. (gradient profile 10-90% (ii) in 25min). The title compound (0.68g) was obtained as a white foam by concentration of the required fraction under reduced pressure and drying by repetitive addition of acetonitrile and concentration under reduced pressure.

Analytical h.p.l.c. (gradient profile 10-90% (ii) in 25min) Rt 26.1min

(h) rel-(3R,3aR,6aS)-2-Oxo-3-[4-(5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-yl)-butyl]-hexahydro-pyrrolo[3,4-b]pyrrole-1,5-dicarboxylic acid 5-benzyl ester 1-methyl ester

A solution of the product of step (g) (0.635g) in THF (25ml) was added to sodium hydride (0.095g, 60% dispersion in oil) at room temperature. After 25 min, methyl chloroformate (0.294ml) was added and stirring continued for 24h. Further sodium hydride (0.095g) followed by methyl chloroformate (0.294ml) were added and stirring continued for 72h. The mixture was poured into phosphate buffer (pH6.5) and extracted with ethyl acetate. The combined, dried (sodium sulfate) organic extracts were concentrated under reduced pressure and the residue was treated with dichloromethane (40ml) and trifluoroacetic acid (6ml). After stirring at room temperature for 4h, the solution was concentrated under reduced pressure and the residue subjected to preparative h.p.l.c. (gradient profile 30-80% (ii) in 15min). The title compound (0.451g) was obtained as a white waxy solid by concentration of the required fraction under reduced pressure and drying by repetitive addition of acetonitrile and concentration under reduced pressure.

Analytical h.p.l.c. (gradient profile 10-90% (ii) in 25min) Rt 22.4min

(i) rel-(3R,3aR,6aS)-3-(4-Carbamimidoyl-butyl)-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-1-carboxylic acid methyl ester (bis)trifluoro-acetate-GR208604A

A mixture of the product of step (h) (0.05g), trifluoroacetic acid (16µl), 10% palladium on carbon (0.034g) and ethyl acetate (40ml) was stirred under an

atmosphere of hydrogen for 24h. The reaction mixture was filtered through Harborlite™ and the filtrate evaporated under reduced pressure to give the title compound (0.15g) as an oil.

Mass spectrum: Found: MH^+ 283

(j) rel-(3R,3aR,6aS)-3-(4-Carbamimidoyl-butyl)-5-(1H-indole-2-carbonyl)-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-1-carboxylic acid methyl ester trifluoro-acetate

A solution of triethylamine (7.0µl) in acetonitrile (1ml) was added dropwise to a stirred solution of the product of step (i) (0.026g) and 1H-indole-2-carbonyl chloride* (0.009g) in acetonitrile (1ml) and stirred at room temperature for 72h. The reaction was concentrated under reduced pressure and the residue subjected to preparative h.p.l.c. (gradient profile 10-90% (ii) in 25min) to give the title compound (0.018g) as a cream coloured solid by concentration of the required fraction under reduced pressure and drying by repetitive addition of acetonitrile and concentration under reduced pressure.

Analytical h.p.l.c. (gradient profile 10-90% (ii) in 25min) Rt 19.8min

Mass spectrum: Found: MH^+ 426

*W. H. Parsons *et al.*, J. Med. Chem., 1989, 32, 1681.

H.P.L.C. Conditions

Preparative high performance liquid chromatography (h.p.l.c.) was carried out using a Dynamax 60A C18 8µM 25cm x 41.4mm i.d. column eluted with a mixture of solvents (i) 0.1% trifluoroacetic acid in water and (ii) 0.05% trifluoroacetic acid in acetonitrile, at a flow rate of 45ml/minute. Analytical h.p.l.c. was carried out using a Dynamax 60A C18 8µM 25cm x 4.6mm i.d. column using eluants as for preparative h.p.l.c. at a flow rate of 1ml/minute.

Compound Examples 4-6 were prepared by a method analogous to that for Compound Example 3:

Compound Example 4

rel-(3R,3aR,6aS)-5-(1H-benzoimidazole-2-sulfonyl)-3-(4-carbamimidoyl-butyl)-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-1-carboxylic acid methyl ester trifluoro-acetate

Mass spectrum: Found: MH^+ 463

Analytical h.p.l.c. (gradient profile 10-90% (ii) in 25min) Rt 18.0min

Compound Example 5

rel-(3R,3aR,6aS)-3-(4-carbamimidoyl-butyl)-5-(naphthalene-1-sulfonyl)-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-1-carboxylic acid methyl ester trifluoro-acetate

Mass spectrum: Found: MH^+ 473

Analytical h.p.l.c. (gradient profile 10-90% (ii) in 25min) Rt 21.95min

Compound Example 6

rel-(3R,3aR,6aS)-3-(4-carbamimidoyl-butyl)-5-(naphthalene-2-sulfonyl)-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-1-carboxylic acid methyl ester trifluoro-acetate

Mass spectrum: Found: MH^+ 473

Analytical h.p.l.c. (gradient profile 10-90% (ii) in 25min) Rt 20.0min

Compound Example 7

rel-(3R,3aR,6aS)-3-(4-Carbamimidoyl-butyl)-5-(1H-indole-2-carbonyl)-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-1-carboxylic acid methylamide trifluoro-acetate

(a) rel-(3R,3aS,6aS)-3-{4-[4-(2-Nitro-benzyl)-5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-yl]-butyl}-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-5-carboxylic acid benzyl ester-

A mixture of the product of Compound Example 3, step (f) (0.2g) *o*-nitrobenzylbromide (0.216g) and triethylamine (0.174ml) in DMF (3ml) was stirred at room temperature for 26h. The reaction mixture was partitioned between pH7.0 buffer and ethyl acetate. The organic phase was separated,

dried (magnesium sulfate) and concentrated under reduced pressure. The crude product was subjected to flash column chromatography eluting with ethyl acetate:methanol (95:5) to give the title compound (0.079g) as a glassy solid.

Mass spectrum: Found: MH^+ 536

(b) rel-(3R,3aS,6aS)-1-Methylcarbamoyl-3-{4-[4-(2-nitro-benzyl)-5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-yl]-butyl}-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-5-carboxylic acid benzyl ester

A solution of the product of step (a) (1.0g) in THF (200ml) at 0°C was treated with sodium hydride (0.37g, 60% dispersion in oil). After 10 min, methyl isocyanate (0.55ml) was added and the mixture reached room temperature over 2h. The mixture was then partitioned between saturated ammonium chloride solution and ethyl acetate. The separated organic phase was washed with brine, dried (magnesium sulfate) and concentrated under reduced pressure. The crude product was subjected to flash column chromatography eluting with chloroform:methanol (30:1) to give the title compound (1.0g) as a pale yellow foam.

T.l.c. (Silica, chloroform:methanol, 15:1) Rf 0.5

(c) rel-(3R,3aS,6aS)-1-Methylcarbamoyl-2-oxo-3-[4-(5-oxo-4,5-dihydro-[1,2,4]oxadiazol-3-yl)-butyl]-hexahydro-pyrrolo[3,4-b]pyrrole-5-carboxylic acid benzyl ester

A solution of the product of step (b) (0.15g) in anhydrous dioxan (120ml) was photolysed for 2h, and then concentrated under reduced pressure. The residue was subjected to flash column chromatography eluting with cyclohexane:ethyl acetate:acetic acid (10:90:1) to give the title compound (0.048g) as an orange solid.

T.l.c. (Silica, ethyl acetate:cyclohexane, 65:35) Rf 0.3

(d) rel-(3R,3aR,6aS)-3-(4-Carbamimidoyl-butyl)-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-1-carboxylic acid methylamide acetate

A mixture of the product of step (c) (0.162g), acetic acid (75µl), 10% palladium on carbon (0.225g) and ethyl acetate (15ml) was stirred under an atmosphere of hydrogen for 24h. The reaction mixture was filtered through harborlite™ and the filtrate evaporated under reduced pressure to give a crude sample of the desired product. Triturating the crude product with ether provided the title compound (0.05g) as a buff solid. Mass spectrum: Found: MH^+ 282

(e) rel-(3R,3aR,6aS)-3-(4-Carbamimidoyl-butyl)-5-(1H-indole-2-carbonyl)-2-oxo-hexahydro-pyrrolo[3,4-b]pyrrole-1-carboxylic acid methylamide trifluoro-acetate

A solution of triethylamine (43µl) in acetonitrile (1ml) was added dropwise to a stirred solution of the product of step (d) (0.025g) and 1H-indole-2-carbonyl chloride (0.010g) in acetonitrile (1ml) and stirred at room temperature for 52h. The reaction was concentrated under reduced pressure and the residue subjected to preparative h.p.l.c. (gradient profile 5-50% (ii) in 10min; 50% (ii) isochratic for 5min) to give the title compound (0.007g) as a cream coloured solid by concentration of the required fraction under reduced pressure and drying by repetitive addition of acetonitrile and concentration under reduced pressure.

Analytical h.p.l.c. (gradient profile 10-90% (ii) in 25min) Rt 20.0min (for HPLC conditions see Compound Example 3) Mass spectrum: Found: MH^+ 425

Biological Data

The results obtained by testing example compounds in the example assays are indicated below:

Compound Example	Example Assay															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14 Plasma	14 Blood	15
1	0.227															
2	0.229															
3		0.011			0.001	1.19	0.019	1.60	0.186		2.6			unstable	<30min	
4		0.278			0.037	0.46	0.005	0.36	0.200		1.1			unstable		
5		0.039			0.004	0.537	0.017	0.033	0.207		0.045					
6		0.007			0.017	0.053	0.015	0.110	0.038							
7		0.271			0.007	11.4			5.8					3.0hr	3.7hr	

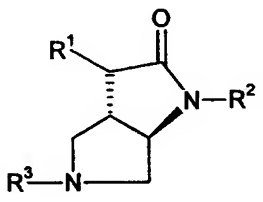
Data in columns 1-13 is indicated at $IC_{50}(\mu M)$

Data in column 14 is indicated as $t_{1/2}$

Claims:

1. An inhibitor of a serine protease enzyme which is a substituted derivative of trans-hexahydropyrrolo[3,4-b]pyrrol-2-one.

2. An inhibitor of a serine protease enzyme which is a compound of formula I:



(I)

(relative stereochemistry indicated)

wherein R^1 is a moiety adapted to fit in the S_1 specificity subsite of the enzyme; R^2 is a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and enzyme kinetic properties of the inhibitor; R^3 is a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and physicochemical properties of the inhibitor; and physiologically acceptable salts and solvates thereof.

3. An inhibitor of a serine protease enzyme according to claim 2, wherein R^2 represents a lactam activating moiety.

4. Use of a substituted derivative of trans-hexahydropyrrolo[3,4-b]pyrrol-2-one as an inhibitor of a serine protease enzyme.

5. An inhibitor according to any one of claims 1 to 3 for use as a pharmaceutical in the treatment of a disease associated with activity of a serine protease enzyme.

6. An inhibitor according to claim 2 or claim 3 wherein R^1 represents C_{2-4} alkyl or C_{2-4} alkenyl for use as an inhibitor of an elastase-like enzyme, e.g. human neutrophil elastase.

7. An inhibitor according to claim 6 wherein R^1 represents propyl or isopropyl.

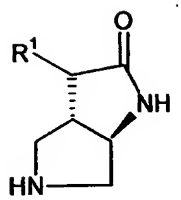
8. An inhibitor according to claim 2 or claim 3 wherein R^1 represents $(CH_2)_{2-4}NHC(=NH)NH_2$, $(CH_2)_{1-2}PhC(=NH)NH_2$, $(CH_2)_{3-5}C(=NH)NH_2$, $CH_2(cyclohexyl)NH_2$, $(CH_2)_{1-3}(NH)_{0-1}Het$ (wherein Het represents a 5 or 6 membered aromatic ring containing 1 or more nitrogen atoms and optionally substituted by amine) or $(CH_2)_{3-5}NH_2$ for use as an inhibitor of a trypsin-like enzyme, e.g. thrombin or tryptase.

9. An inhibitor according to claim 2 or claim 3 wherein R^1 represents benzyl for use as an inhibitor of a chymotrypsin-like enzyme, e.g. Cathepsin G.

10. An inhibitor according to claim 2, 3, 6 or 7 wherein R^2 represents CHO or SO_2C_{1-6} alkyl.

11. An inhibitor according to claim 2, 3 or 8 wherein R^2 represents $CONH(CH_2)_{1-4}Ph$, $SO_2(CH_2)_{0-1}Ph$, $COOC_{1-4}$alkyl or $CONHC_{1-4}$alkyl.

12. A compound of formula II

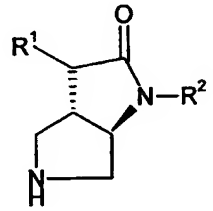


(II)

(relative stereochemistry indicated)

wherein R^1 is a moiety adapted to fit in the S_1 specificity subsite of the enzyme.

13. A compound of formula II'



(II)'

(relative stereochemistry indicated)

wherein R^1 is a moiety adapted to fit in the S_1 specificity subsite of the enzyme; R^2 is a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and enzyme kinetic properties of the inhibitor.

14. A compound according to claim 12 or claim 13 wherein R^1 represents $(CH_2)_{2-4}NHC(=NH)NH_2$, $(CH_2)_{1-2}PhC(=NH)NH_2$, $(CH_2)_{3-5}C(=NH)NH_2$, $CH_2(cyclohexyl)NH_2$, $(CH_2)_{1-3}(NH)_{0-1}Het$ (wherein Het represents a 5 or 6 membered aromatic ring containing 1 or more nitrogen atoms and optionally substituted by amine) or $(CH_2)_{3-5}NH_2$.

15. A compound according to claim 12 or claim 13 wherein R^1 represents C_{2-4}alkyl or C_{2-4}alkenyl.

16. A compound according to any one of claims 13 or 15 wherein R^2 represents CHO or SO_2C_{1-6}alkyl.

17. A compound according to any one of claims 13 or 14 wherein R^2 represents $CONH(CH_2)_{1-4}Ph$, $SO_2(CH_2)_{0-1}Ph$, $COOC_{1-4}$alkyl or $CONHC_{1-4}$alkyl.

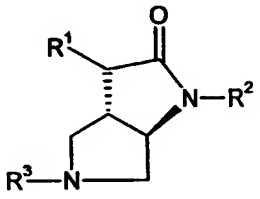
18. A method of treatment of chronic bronchitis or ARDS which comprises administering to a patient an effective amount of a neutrophil elastase inhibitor according to claim 6, 7 or 10.

19. A method of treatment of diseases of the vascular system especially thrombosis which comprises administering to a patient an effective amount of a thrombin inhibitor according to claim 8 or 11.

20. A method of treatment of asthma which comprises administering to a patient an effective amount of a tryptase inhibitor according to claim 8 or 11.

21. A library comprising a plurality of substituted derivatives of trans-hexahydropyrrolo[3,4-b]pyrrol-2-one.

22. A library comprising a plurality of compounds of formula I



(I)

(relative stereochemistry indicated)

wherein R^1 is a moiety adapted to fit in the S_1 specificity subsite of the enzyme; R^2 is a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and enzyme kinetic properties of the inhibitor;

R^3 is a moiety adapted to optimise the potency, pharmacokinetics, pharmacodynamics, selectivity and physicochemical properties of the inhibitor; and pharmaceutically acceptable salts and solvates thereof.

23. A library according to claim 21 or 22 comprising at least 10 different compounds.

24. A library according to claim 22 or claim 23 wherein R_1 represents C_{2-4}alkyl or C_{2-4}alkenyl.

25. A library according to claim 22 or claim 23 wherein R_1 represents $(CH_2)_{2-4}NHC(=NH)NH_2$, $(CH_2)_{1-2}PhC(=NH)NH_2$, $(CH_2)_{3-5}C(=NH)NH_2$, CH_2(cyclohexyl)NH_2, $(CH_2)_{1-3}(NH)_{0-1}$Het (wherein Het represents a 5 or 6 membered aromatic ring containing 1 or more nitrogen atoms and optionally substituted by amine) or $(CH_2)_{3-5}NH_2$.

26. A library according to any one of claims 21 to 25 which is a solid phase library.

27. A library according to any one of claims 21 to 25 which is a solution phase library.

28. A library according to claim 26 or 27 which is a discrete library.

29. A library according to claim 26 or 27 which is a pooled library.

30. Use of a library according to any one of claims 21 to 24 for screening for an inhibitor of neutrophil elastase.

31. Use of a library according to any one of claims 21, 22, 23 and 25 for screening for an inhibitor of thrombin or tryptase.

32. A method of screening for an inhibitor of a serine protease enzyme which comprises treating a serine protease enzyme with an inhibitor according to any one of claim 1 to 3 and determining the extent to which inhibition has occurred.

33. A method of identifying an inhibitor of a serine protease enzyme which comprises:

(a) preparation of a number of substituted derivatives of trans-hexahydropyrrolo[3,4-b]pyrrol-2-one;

(b) treatment of a sample of the enzyme in question with a sample of each of the derivatives so prepared; and

(c) determining the extent to which inhibition of the enzyme has occurred.

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/EP 98/05744

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D489/04 C07B61/00 A61K31/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C07B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 98 43975 A (GLAXO GROUP LIMITED) 8 October 1998 see the whole document	1
A,P	WO 97 48706 A (WARNER-LAMBERT COMPANY) 24 December 1997 see page 5, line 25 - page 6	1
A	WO 95 24186 A (PHARMACOPEIA INC.) 14 September 1995 see page 1 - page 6, line 20	21,30,31
A	WO 95 03278 A (ZAIDAN HOJIN BISEIBUTSU KAGAKU KENKYUKAI ET AL.) 2 February 1995 see claim 1	1

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 February 1999	02/03/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kyriakakou, G

3

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/EP 98/05744

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SPENCER KNAPP ET AL.: "Synthesis and reactions of Iodo Lactams" JOURNAL OF ORGANIC CHEMISTRY, vol. 53, no. 17, 1988, pages 4006-4014, XP002093059 see page 4009; examples 13-15; table II	1

3

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 2,13,22,31

A reasonable search was impossible for the claims 2,13,22,31 since a practically unlimited number of chemical compounds would have to be considered. More specifically the definition of substituents R1, R2 and R3 is vague and unclear.e.g R1 is a moiety tofit in the S1 subsite of the enzyme or R2 and R3 are moieties to optimise the potency, pharmacokinetics etc of the compound.
The search was therefore limited to the general concept, the examples and the compounds disclosed in some of the claims.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter ional Application No
PCT/EP 98/05744

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9843975 A	08-10-1998	AU 6926198 A	22-10-1998
WO 9748706 A	24-12-1997	AU 3232597 A	07-01-1998
WO 9524186 A	14-09-1995	AU 690656 B	30-04-1998
		AU 1999195 A	25-09-1995
		CA 2183428 A	14-09-1995
		EP 0751765 A	08-01-1997
		JP 9510442 T	21-10-1997
		US 5618825 A	08-04-1997
		US 5756810 A	26-05-1998
WO 9503278 A	02-02-1995	NONE	